NOAA-NATIONAL OCEAN SERVICE

CENTER FOR COASTAL ENVIRONMENTAL HEALTH AND BIOMOLECULAR RESEARCH

FY01 SIGNIFICANT ACCOMPLISHMENTS

MARINE BIOTOXINS PROGRAM

CDNA LIBRARY FOR STUDIES ON FUNCTIONAL GENOMICS OF FLORIDA RED TIDE DINOFLAGELLATE, *KARENIA BREVIS*

Understanding mechanisms controlling cell proliferation and toxicity of dinoflagellates is critical to the development of predictive indicators for the physiological status of blooms and identification of potential targets for species-specific control measures. Progress in this field is hampered by the overwhelming lack of knowledge of the genetic make-up of dinoflagellates: currently there are less than 50 expressed gene sequences for dinoflagellates in the NIH GenBank Database. Sequencing of a cDNA library, developed for the Florida red tide dinoflagellate, *Karenia brevis* (formerly *Gymnodinium breve*), was undertaken this year to help fill this void. Expressed sequence tags (EST) will be deposited in the dbEST database of GenBank and will be used for developing microarrays for assessing dinoflagellate gene expression.

MICROBIAL COMMUNITIES CAN PROTECT HAB SPECIES AGAINST ALGAE-KILLING BACTERIA

As part of an investigation into potential strategies for mitigating the negative consequences of HABs, we are studying the role of bacteria as natural growth regulators of HAB species. We have discovered two algicidal (algae killing) bacteria targeting the Florida red tide dinoflagellate, *Karenia brevis*. These bacteria are able to rapidly lyse most *K. brevis* cultures, yet it appears that certain strains are resistant to algicidal attack. Recent experiments have demonstrated that this apparent resistance is due to the inhibition of algicidal bacteria growth by the microbial community associated with resistant *K. brevis* strains. Remarkably, both resistance as well as susceptibility to algicidal activity can be transferred between *K. brevis* cultures with the exchange of their respective unattached bacterial assemblages. Thus, it is clear if algicidal bacteria play a significant role in regulating HAB dynamics and are to be considered as a part of a possible control strategy, interactions with the ambient microbial community will play a crucial role in determining the effect of such bacteria on algal growth.

TRACKING DOMOIC ACID ASSOCIATED WITH TOXIC *PSEUDO-NITZSCHIA* BLOOMS

Over the past decade, the Monterey Bay, CA ecosystem has experienced a variety of faunal mortality events associated with the marine algal toxin, domoic acid (DA), and diatoms of the genus *Pseudo-nitzschia* that produce it. Through a collaborative study with colleagues at the Monterey Bay Aquarium Research Institute, we have begun to track DA associated with *Pseudo-nitzschia* bloom events through the horizontal and vertical mapping of particulate and dissolved toxin as determined by receptor binding assay. We have learned that particulate and dissolved forms of DA can occur throughout the vertical water column, thereby potentially exposing animals in both pelagic and benthic communities to this toxin. Moreover, toxin distributions as well as the proportions of particulate and dissolved DA do appear to change with the progression of a bloom. These findings represent the first steps toward developing models of how DA distributions vary during *Pseudo-nitzschia* blooms, which is essential for predicting routes of toxin trophic transfer and thus implications for not only public health, but also the health of ecosystems and their trophic structure.

INITIATION OF THE SOUTH CAROLINA PHYTOPLANKTON MONITORING NETWORK

The inaugural year for South Carolina Phytoplankton Monitoring Network began with great enthusiasm and the opening of a new home page http://www.chbr.noaa.gov/CoastalResearch/SCPMN/SCPMNmain.htm. This community outreach p rogram consists of high school marine science and biology classes monitoring local waters for the presence of possible harmful algal species. Teachers participating in the network attended a workshop on algal identification and sampling techniques. Currently, 12 teachers and approximately 170 students are actively sampling local waters for harmful algae. Based on the observations of these groups, a number of potentially harmful species have been detected in South Carolina, some for the first time. These include representatives of the genera *Prorocentrum*, *Pseudo-nitzschia*, *Heterosigma*, and *Akashiwo*. Additional community groups will be added to the network during the next year to extend coverage of this program along the coast of South Carolina.

PURIFICATION OF A HYDROPHILIC TOXIN FROM *PFIESTERIA PISCICIDA*

The uncharacterized toxic substances associated with *Pfiesteria piscicida* represent a difficult challenge for health officials. These toxins have been implicated in massive marine mortalities of fish and other wildlife in a number of estuarine systems, and have also been implicated in serious human health related incidents. Without toxin structure and preparative quantities of the toxin itself, very little can be done to mitigate the toxic effects and/or protect human health. This past year we have isolated a polar (water-soluble) toxin from *Pfiesteria piscicida*. This semi-purified material from polar, water-soluble extracts, although not yet analytically pure, is highly cytotoxic and tests positive for the induction of c-fos reporter gene activity, a response mediated by P2X-7 receptor pathways. This information is useful in designing more efficient monitoring techniques as well as providing insights into the toxic substance's mode of action. Mass culture and preparative toxin production will continue.

BREVETOXIN-2 PRODUCTION AND PURIFICATION FOR METABOLISM/ DETECTION RESEARCH

The lack of toxin standards for use in research has always been a problem for the health community. Without well-characterized and highly purified toxin standards or reference materials, it is difficult or impossible to probe a toxin's mode of action, metabolism, or natural degradation. Such data are needed to support research aimed at mitigating toxin exposures and toxic effects. During this past fiscal year, we have developed a chromatographic methodology to produce PbTx2 as determined by MS and NMR, and have provided four milligrams of highly purified PbTx2 to NOS as well as our FDA and EPA partners. With purified toxin in hand, NOS and its partners can now label this toxin with radioactive tritium (3H) and/or a stable isotope, deuterium (2H), for use in monitoring toxin metabolism and degradation within living systems. The deuterated toxin will also be used by NOS and its partners to develop accurate, highly sensitive detection methodologies using mass spectrometry (MS) and nuclear magnetic resonance spectroscopy (NMR).

TOXICOGENOMICS: A GLOBAL APPROACH TO ASSESSING MARINE TOXIN EXPOSURE AND EFFECTS

Toxin exposure almost always causes changes in gene expression, either directly, due to the specific interaction of a toxic agent with its receptor, or indirectly due to the induction of intracellular signaling cascades. Toxicogenomics is the application of DNA arrays to identify a specific pattern of gene expression induced by a particular toxicant. Once a "signature" gene response is identified, this information may be useful for elucidating a toxic mode of action and may potentially yield biomarkers of exposure unique for a particular toxicant or class of toxicants. This year the Marine Biotoxins Program co-organized a workshop on "Toxicogenomics and Nanotechnologies: New Frontiers for Mycotoxins and Phycotoxins" (June 22-23, 2001; Tufts University Bedford, MA) and carried out preliminary studies to determine the suitability of this approach for algal toxin exposure. Changes in gene expression in brains and livers of mice exposed to brevetoxin were studied. Several genes were found to be induced in response to this toxin class. Ongoing studies will determine the dose/response and time course of genetic responses and compare gene induction "signatures" of different algal toxin classes.

THE PUTATIVE *PFIESTERIA* TOXIN TARGETS IMMUNE CELLS TO RELEASE PROINFLAMMATORY CYTOKINES

The identification of P2X-7 receptors as a target for the putative *Pfiesteria* toxin led to the unexpected implication of the putative *Pfiesteria* toxin in immune function. P2X-7 receptors are largely restricted to cells of immune origin. This has lead to investigations employing two additional models, macrophages and microglia (macrophages of the brain). This past years work indicates that the putative *Pfiesteria* toxin induces the release of the proinflammatory cytokine interleukin 1b from macrophages. This cytokine is responsible for initiating a cascade of responses leading to inflammation. The hypothesis we are now testing is that the putative *Pfiesteria* toxin disrupts normal immune function. Such a disruption leads to a chronic inflammatory response that is manifest in granulomatous lesions, which is a common response observed in fish exposed to *Pfiesteria*. If this response becomes systemic, it may lead to a septic shock-like effect resulting in massive fish kills.

JOINT RUSSIAN-US SURVEY OF THE BLACK SEA IDENTIFIES *PROROCENTRUM LIMA*

A joint US-Russian collaboration sponsored by the US Civilian Research and Development Foundation identified a HAB species responsible for diarrheic shellfish poisoning in the Black Sea. Phytoplankton and shellfish were collected throughout the entire Russian coastline of the Black Sea by a joint US-Russian team comprised of scientists from CCEHBR, the Shirshov Institute of Oceanology, and Moscow State University. The dinoflagellate *Prorocentrum lima*, which produces okadaic acid, was identified growing in association with macroalgae, which dominates many parts of the subtidal zone. *P. lima* appeared to have a substrate preference in favor of *Dictyota dichotoma* and *Padina pavonica*, both species non-endemic to the Black Sea, as compared to the dominant indigenous macrophyte species *Cystoseira barbata* and *Cladophora albida*. *P. lima* cells were also identified in mussel stomachs by microscopic examination. Other potentially toxic phytoplankton species (*Dinophysis* and *Pseudonitzschia*) were also observed in low numbers. Further analysis of toxin burden in mussels collected from the Utrish Center for Marine Biotechnology and Aquaculture will be carried out at the Charleston laboratory. Enumeration of preserved phytoplankton communities samples will be examined by Russian partner scientists. Although many toxin producing dinoflagellates are known to be present in Russian coastal waters, there are no regulations regarding contamination in seafood and no statistics available on human poisonings.